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Poster P5

HINT1 deficiency impairs actin cytoskeleton and calcium signaling

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Loss of functional histidine triad nucleotide-binding protein 1 (HINT1) causes a rare form of inherited peripheral neuropathy with neuromyotonia (NMAN). Patients suffer from motor-greater-than-sensory polyneuropathy with an age of onset within the first decade of life. HINT1 is an ubiquitously expressed purine phosphoramidase that functions as an inhibitor of pro-oncogenic transcription factors, and acts as an adaptor protein of the endocannabinoid signaling pathway in the central nervous system (CNS). Yet, HINT1's role in the peripheral nerves remains uncharacterized.

Currently, 28 NMAN-causing variants have been described resulting in loss of HINT1 function, e.g. by impairing conformational stability and leading to protein degradation (R37P) or abolishing enzymatic activity without affecting protein stability (H112N). We genetically engineered HeLa cell lines deficient for HINT1 using the CRISPR/Cas9 editing technology and studied their transcriptome profile. Gene ontology and pathway analysis identified actin cytoskeleton remodeling and integrin signaling as affected pathways. Further validation of misregulated genes involved in intracellular signaling pathways showed downregulation of calcium-related proteins in particular. Using our HINT1-KO HeLa model and NMAN patient-derived fibroblasts homozygous for the R37P or H112N variants, we functionally validated the effect of HINT1 deficiency on the actin cytoskeleton dynamics using a wound healing assay. We found mobility and attachment impediments in the HINT1 deficient cells. Additionally, we showed HINT1-associated effect on IP3-mediated Ca2+ response that depends on HINT1 protein abundance and the nature of applied stimuli. Our findings identify and characterize two functionally related affected cellular pathways as a result of loss of HINT1 that might underlie the NMAN disease mechanism.